

10/574625

IAP20 Rec'd PCT/PTO 04 APR 2006

SEQUENCE LISTING

<110> Hoff, Tine  
<120> A Method of Screening For Protein Secreting Recombinant Host Cells  
<130> 10355.204-US  
<160> 11  
<170> PatentIn version 3.3  
<210> 1  
<211> 2349  
<212> DNA  
<213> Bacillus subtilis  
  
<220>  
<221> YkdA\_protein  
<222> (1000)..(2349)  
<223> 1-999 is promoter  
  
<220>  
<221> CDS  
<222> (1000)..(2349)  
  
<400> 1  
tcttcaagg attcatgttt gtttaccaac ctgttctgtta agcagttcat attttctcag 60  
ggttcttc aataacctcat caaaaacgtc cggcacagag gcgtgtatca cctcagctcc 120  
ctctcccggtt attccgcctt tggttgcaac acgttccagc gtctcctcga aagacatatt 180  
tttttcgatc agcatcttgc cagttccgta tagcgaatga atcagaaaaat caaaggcttc 240  
ttctttggac aggctgctgt ttctgacggc agacagtgcc agttttcaa agattgcagc 300  
tatgaatccc ggtgccgagc tcgttaaatt gctggccaca tctaaattcg attcttgat 360  
ttccccgtaca cggctgaaaa ccgataacaa ttcattcaga cggtctttt tctctgcagc 420  
cagtgcgtca ctgtgaacga caagttagat gccggcttct gcttcggacg taatggcagg 480  
aataaacacgt gagataccgg cttctgtttc tgcctccaaa agacgcagcg gcacaccggc 540  
agctatggat acgatgtgag tatttctgtt cacatacggg tacagacggc gcattgttc 600  
gatgacatgg agtggcgaaa cgcatatcaa aatcaattgg cacgtatttgc cccattctc 660  
caacggatca gccgatacgt ttggataatc tgacatgagt gcccgcagcc gctccccctt 720  
cggtctcggtc tcaataaata gctcattctc ttttatttgt tcatgtttca acagctgtct 780  
agcgatcata tccgccatgc tgccatatcc aatcaatcca atctgttcca tcgactcagt 840

cctttcatat acaatatgaa gtgtaccgtt ttccgcactt tttcacaatt tcccataatc	900
tttcatttt tatccccacag tttttgttta tgataaaactc aagtataaaa cctatcaata	960
taaatagaca tgtgaaaata gagaaacgga gtgaacatg atg gat aac tat cgt Met Asp Asn Tyr Arg 1 5	1014
gat gaa aac aga acg aaa ggt aat gag aat gag gtc ttt tta acg aaa Asp Glu Asn Arg Thr Lys Gly Asn Glu Asn Glu Val Phe Leu Thr Lys 10 15 20	1062
gag aac gat cag agc gcc tcc tac tcg gcc cgcc aat gtc att cat gat Glu Asn Asp Gln Ser Ala Ser Tyr Ser Ala Arg Asn Val Ile His Asp 25 30 35	1110
cag gag aag aaa aaa cga gga ttc gga tgg ttc aga ccg ttg ctt ggc Gln Glu Lys Lys Lys Arg Gly Phe Gly Trp Phe Arg Pro Leu Leu Gly 40 45 50	1158
gga gtg atc ggc ggc agt ctt gct ctt ggc att tac acg ttt aca ccg Gly Val Ile Gly Gly Ser Leu Ala Leu Gly Ile Tyr Thr Phe Thr Pro 55 60 65	1206
ctt ggt aac cat gat tct cag gac act gca aaa caa tca tcc agc cag Leu Gly Asn His Asp Ser Gln Asp Thr Ala Lys Gln Ser Ser Gln 70 75 80 85	1254
cag caa acg caa tct gtt aca gca aca agc acc tcc tct gaa tct aaa Gln Gln Thr Gln Ser Val Thr Ala Thr Ser Thr Ser Ser Glu Ser Lys 90 95 100	1302
aaa agc tca agc agc tca tct gca ttc aag agc gag gac tct tct aaa Lys Ser Ser Ser Ser Ala Phe Lys Ser Glu Asp Ser Ser Lys 105 110 115	1350
atc tca gat atg gta gaa gac ctt tca cca gcg att gtc ggt att aca Ile Ser Asp Met Val Glu Asp Leu Ser Pro Ala Ile Val Gly Ile Thr 120 125 130	1398
aat ctt cag gca caa tca aac agc tct ttg ttc ggc tct agt tct tct Asn Leu Gln Ala Gln Ser Asn Ser Ser Leu Phe Gly Ser Ser Ser Ser 135 140 145	1446
gat tcc agc gaa gat aca gaa agc ggt tca ggg tca ggt gtc att ttc Asp Ser Ser Glu Asp Thr Glu Ser Gly Ser Gly Ser Gly Val Ile Phe 150 155 160 165	1494
aaa aaa gag aat ggc aag gct tat atc att aca aat aac cac gtc gta Lys Lys Glu Asn Gly Lys Ala Tyr Ile Ile Thr Asn Asn His Val Val 170 175 180	1542
gaa ggg gca tca tca ctg aag gta tct tta tat gac ggc act gag gtt Glu Gly Ala Ser Ser Leu Lys Val Ser Leu Tyr Asp Gly Thr Glu Val 185 190 195	1590
act gca aag ctg gta ggc agt gac tcg tta act gat tta gcc gtc ctc	1638

Thr Ala Lys Leu Val Gly Ser Asp Ser Leu Thr Asp Leu Ala Val Leu			
200	205	210	
caa atc agt gat gac cac gtc aca aaa gtg gca aac ttc ggt gat tca			1686
Gln Ile Ser Asp Asp His Val Thr Lys Val Ala Asn Phe Gly Asp Ser			
215	220	225	
tct gat ctt aga aca ggc gag acc gtt att gcg att ggg gat ccg ctt			1734
Ser Asp Leu Arg Thr Gly Glu Thr Val Ile Ala Ile Gly Asp Pro Leu			
230	235	240	245
gga aaa gac ctg tcc cgc aca gta aca caa gga att gta agc ggc gtg			1782
Gly Lys Asp Leu Ser Arg Thr Val Thr Gln Gly Ile Val Ser Gly Val			
250	255	260	
gac aga acg gtt tca atg tct aca tca gcc ggc gaa acg agc att aac			1830
Asp Arg Thr Val Ser Met Ser Thr Ser Ala Gly Glu Thr Ser Ile Asn			
265	270	275	
gtc att cag aca gac gca gca att aat cca ggt aac agc ggc ggt cct			1878
Val Ile Gln Thr Asp Ala Ala Ile Asn Pro Gly Asn Ser Gly Gly Pro			
280	285	290	
ttg tta aat aca gac ggc aaa att gtc ggc att aac agt atg aaa atc			1926
Leu Leu Asn Thr Asp Gly Lys Ile Val Gly Ile Asn Ser Met Lys Ile			
295	300	305	
agt gag gat gat gtt gag ggt atc gga ttc gcc att cca agc aat gac			1974
Ser Glu Asp Asp Val Glu Gly Ile Gly Phe Ala Ile Pro Ser Asn Asp			
310	315	320	325
gta aaa ccg att gct gaa gaa ttg ctg tct aaa gga caa att gaa cgt			2022
Val Lys Pro Ile Ala Glu Glu Leu Leu Ser Lys Gly Gln Ile Glu Arg			
330	335	340	
cca tat atc ggt gtc agc atg ctt gat cta gag caa gtg ccg caa aat			2070
Pro Tyr Ile Gly Val Ser Met Leu Asp Leu Glu Gln Val Pro Gln Asn			
345	350	355	
tac caa gaa ggc aca ctc ggc ctg ttc ggc agc cag ctg aat aaa ggc			2118
Tyr Gln Glu Gly Thr Leu Gly Leu Phe Gly Ser Gln Leu Asn Lys Gly			
360	365	370	
gtt tac atc cgt gag gtc gct tca ggc tct cct gct gaa aag gcc gga			2166
Val Tyr Ile Arg Glu Val Ala Ser Gly Ser Pro Ala Glu Lys Ala Gly			
375	380	385	
tta aaa gcg gag gat att atc atc ggc cta aaa ggt aaa gaa att gat			2214
Leu Lys Ala Glu Asp Ile Ile Gly Leu Lys Gly Lys Glu Ile Asp			
390	395	400	405
aca ggc agt gaa ttg cgc aat atc tta tat aaa gac gca aag atc ggt			2262
Thr Gly Ser Glu Leu Arg Asn Ile Leu Tyr Lys Asp Ala Lys Ile Gly			
410	415	420	
gat acc gtt gaa gtg aaa att ctc cga aac ggc aaa gaa atg acg aaa			2310
Asp Thr Val Glu Val Lys Ile Leu Arg Asn Gly Lys Glu Met Thr Lys			

425

430

435

aaa att aaa ctg gat caa aaa gaa gag aaa act tcg taa  
Lys Ile Lys Leu Asp Gln Lys Glu Glu Lys Thr Ser  
440 445

2349

<210> 2  
<211> 449  
<212> PRT  
<213> Bacillus subtilis

<400> 2

Met Asp Asn Tyr Arg Asp Glu Asn Arg Thr Lys Gly Asn Glu Asn Glu  
1 5 10 15

Val Phe Leu Thr Lys Glu Asn Asp Gln Ser Ala Ser Tyr Ser Ala Arg  
20 25 30

Asn Val Ile His Asp Gln Glu Lys Lys Lys Arg Gly Phe Gly Trp Phe  
35 40 45

Arg Pro Leu Leu Gly Gly Val Ile Gly Gly Ser Leu Ala Leu Gly Ile  
50 55 60

Tyr Thr Phe Thr Pro Leu Gly Asn His Asp Ser Gln Asp Thr Ala Lys  
65 70 75 80

Gln Ser Ser Ser Gln Gln Thr Gln Ser Val Thr Ala Thr Ser Thr  
85 90 95

Ser Ser Glu Ser Lys Lys Ser Ser Ser Ser Ser Ala Phe Lys Ser  
100 105 110

Glu Asp Ser Ser Lys Ile Ser Asp Met Val Glu Asp Leu Ser Pro Ala  
115 120 125

Ile Val Gly Ile Thr Asn Leu Gln Ala Gln Ser Asn Ser Ser Leu Phe  
130 135 140

Gly Ser Ser Ser Ser Asp Ser Ser Glu Asp Thr Glu Ser Gly Ser Gly  
145 150 155 160

Ser Gly Val Ile Phe Lys Lys Glu Asn Gly Lys Ala Tyr Ile Ile Thr  
165 170 175

Asn Asn His Val Val Glu Gly Ala Ser Ser Leu Lys Val Ser Leu Tyr  
180 185 190

Asp Gly Thr Glu Val Thr Ala Lys Leu Val Gly Ser Asp Ser Leu Thr  
195 200 205

Asp Leu Ala Val Leu Gln Ile Ser Asp Asp His Val Thr Lys Val Ala  
210 215 220

Asn Phe Gly Asp Ser Ser Asp Leu Arg Thr Gly Glu Thr Val Ile Ala  
225 230 235 240

Ile Gly Asp Pro Leu Gly Lys Asp Leu Ser Arg Thr Val Thr Gln Gly  
245 250 255

Ile Val Ser Gly Val Asp Arg Thr Val Ser Met Ser Thr Ser Ala Gly  
260 265 270

Glu Thr Ser Ile Asn Val Ile Gln Thr Asp Ala Ala Ile Asn Pro Gly  
275 280 285

Asn Ser Gly Gly Pro Leu Leu Asn Thr Asp Gly Lys Ile Val Gly Ile  
290 295 300

Asn Ser Met Lys Ile Ser Glu Asp Asp Val Glu Gly Ile Gly Phe Ala  
305 310 315 320

Ile Pro Ser Asn Asp Val Lys Pro Ile Ala Glu Glu Leu Leu Ser Lys  
325 330 335

Gly Gln Ile Glu Arg Pro Tyr Ile Gly Val Ser Met Leu Asp Leu Glu  
340 345 350

Gln Val Pro Gln Asn Tyr Gln Glu Gly Thr Leu Gly Leu Phe Gly Ser  
355 360 365

Gln Leu Asn Lys Gly Val Tyr Ile Arg Glu Val Ala Ser Gly Ser Pro  
370 375 380

Ala Glu Lys Ala Gly Leu Lys Ala Glu Asp Ile Ile Ile Gly Leu Lys  
385 390 395 400

Gly Lys Glu Ile Asp Thr Gly Ser Glu Leu Arg Asn Ile Leu Tyr Lys  
405 410 415

Asp Ala Lys Ile Gly Asp Thr Val Glu Val Lys Ile Leu Arg Asn Gly  
420 425 430

Lys Glu Met Thr Lys Lys Ile Lys Leu Asp Gln Lys Glu Glu Lys Thr  
435 440 445

Ser

<210> 3  
<211> 8  
<212> DNA  
<213> Artificial

<220>  
<223> octameric motif

<220>  
<221> octameric\_motif  
<222> (1)..(8)

<400> 3  
ttttcata

8

<210> 4  
<211> 44  
<212> DNA  
<213> Artificial

<220>  
<223> Primer

<220>  
<221> Primer\_oth48  
<222> (1)..(44)

<400> 4  
gttcatcgat cgcatggct aatcagacca cttcggtga aggc

44

<210> 5  
<211> 50  
<212> DNA  
<213> Artificial

<220>  
<223> Primer

```

<220>
<221> Primer_oth50
<222> (1)..(50)

<400> 5
ggagcggatt gaacatgcga ttaaatatcc ttcgagacat tttcgatcgc      50

<210> 6
<211> 21
<212> DNA
<213> Artificial

<220>
<223> Primer

<220>
<221> Primer_260558
<222> (1)..(21)

<400> 6
gagtatcgcc agtaaggggc g      21

<210> 7
<211> 44
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 7
gccttcaccc gaagtggtct gattagccga tgcgatcgat gaac      44

<210> 8
<211> 23
<212> DNA
<213> Artificial

<220>
<223> Primer

<220>
<221> Primer_260559
<222> (1)..(23)

<400> 8
gcagccctaa aatcgataa agc      23

<210> 9

```

```

<211> 50
<212> DNA
<213> Artificial

<220>
<223> Primer

<220>
<221> Primer_oth51
<222> (1)..(50)

<400> 9
gcatcgaaa atgtctcgaa ggatatttaa tcgcgttcc aatccgctcc      50

<210> 10
<211> 28
<212> DNA
<213> Artificial

<220>
<223> Primer

<220>
<221> Primer_YKDA6
<222> (1)..(28)

<400> 10
gcgaattcta aactcaagtc ataaacct      28

<210> 11
<211> 26
<212> DNA
<213> Artificial

<220>
<223> Primer

<220>
<221> Primer_YKDAP1
<222> (1)..(26)

<400> 11
gcggatccga tcatgtatgc cattgc      26

```